UTR Reporter Vectors and Viruses

3' UTR, 5' UTR, Promoter Reporter *(Version 1)*

Applied Biological Materials Inc.
#1-3671 Viking Way
Richmond, BC
V6V 2J5 Canada

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Technical Support

For any inquiries about **abm** products and their applications, please contact:

For General Information: info@abmGood.com
To Order Products: order@abmGood.com
For Technical Support: technical@abmGood.com

Toll Free: 1-866-757-2414 (8:30 AM to 5 PM PST, Monday to Friday)

Website: www.abmGood.com

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A. Introduction

Every gene has a 5' and 3' UTR (Untranslated Region) upstream and downstream, respectively, of the coding region. These UTRs are transcribed and remain present in mature mRNA post-processing, but are not present in the final amino acid sequence. Despite their lack of coding ability, the UTRs of a gene are extremely important for regulation of that gene.

The 5' UTR includes the promoter region, which binds the ribosome and initiates translation. The 5' UTR performs other regulatory functions by binding RNA-binding proteins or, in some cases, microRNAs (miRNAs). The 3' UTR's regulatory function is primarily carried out by binding miRNAs, causing translational repression.

Abm offers 3 types of UTR vectors to cover all your potential needs. Our 3' UTR Reporter vectors are ideal for determining the effects of miRNA on the 3' UTR of a region. Our Promoter Reporter constructs are perfect for studying the effects of promoter variants or different conditions on protein translation. Also, **abm** now offers custom 5' UTR Reporter Vectors, which form a hybrid transcript of the 5' UTR fused to a downstream reporter. These vectors are ideal for discovering the effects of RNA binding proteins on translation.

All of **abm**'s UTR reporter vectors are available as ready-to-use DNA plasmids, or packaged into lentiviral particles. Our UTR reporter vectors can be used for direct transfection, causing transient expression. Lentiviral particles allow difficult-to-transfect cells, such as primary cells, to be efficiently transduced for stable, long term expression.

Each UTR sequence is amplified by PCR using a high fidelity Taq polymerase based on the listed NCBI reference sequence provided. The amplified UTR fragment is then sub-cloned into the appropriate vector. The final constructs then undergo sequencing QC, to ensure the accuracy of the UTR insert.

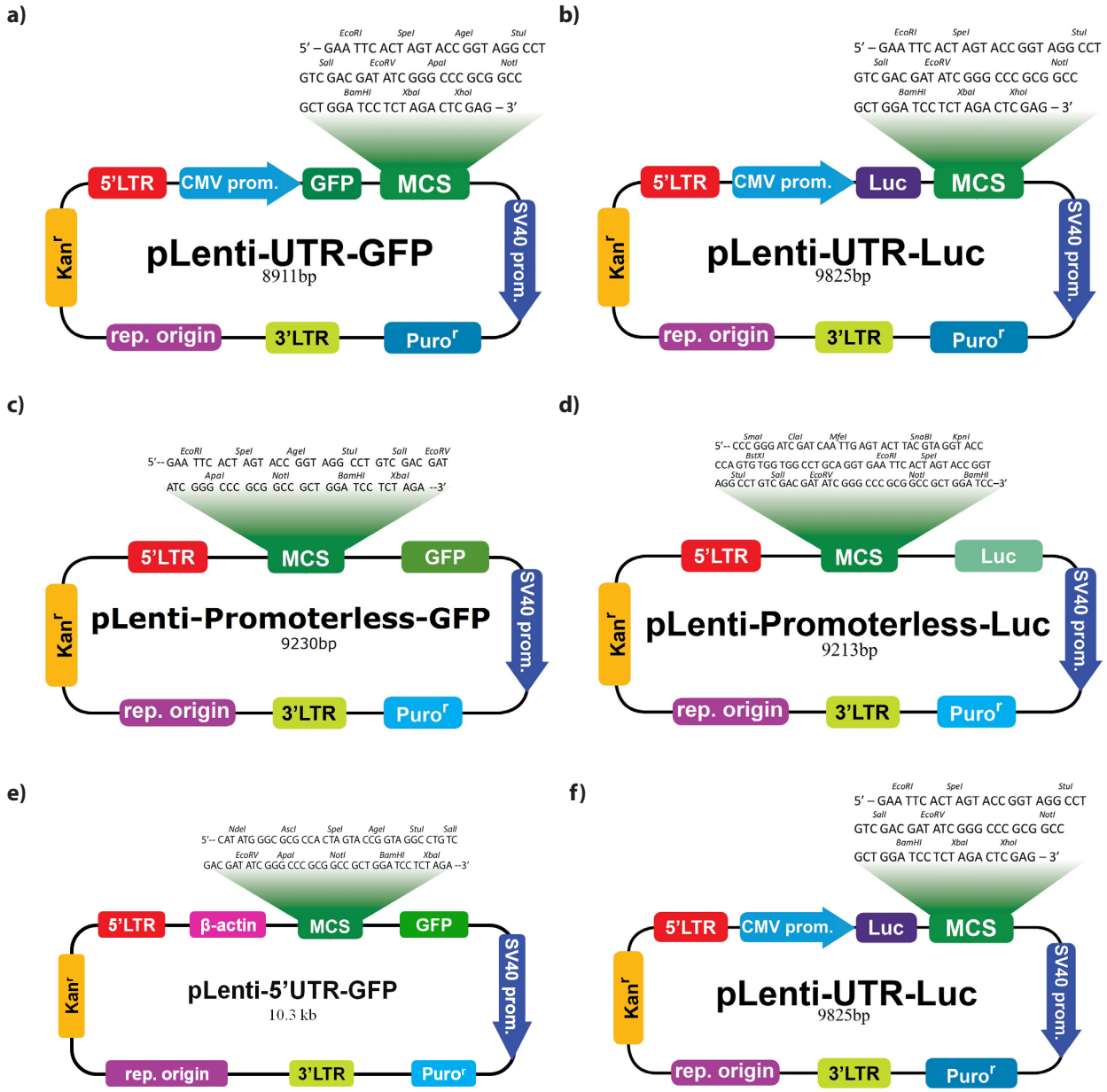

Figure 1: Vector maps of **abm**'s **(a)** 3′ UTR GFP Reporter, **(b)** 3′ UTR Luc Reporter, **(c)** Promoter GFP Reporter, **(d)** Promoter Luc Reporter, **(e)** 5′ UTR GFP Reporter, and **(f)** 5′ UTR Luc Reporter. The UTR of interest is cloned into the Multiple Cloning Site (MCS).

B. UTR Reporter Vectors

i. 3'UTR Reporter Vectors

MicroRNAs (miRNAs) are critical regulators for target mRNA stability and translation (Bartel, DP. 2004 and 2009). Interactions among miRNAs and their target mRNA transcripts are still relatively unknown for most genes. Though computational prediction tools are helpful, a definitive relation has to be determined by experimental assays. 3'-Luc/GFP-UTR reporters have been shown by many researchers to validate these interactions between miRNAs and target gene 3'-UTR regions via standard methods (Lai, EC. 2002; Petersen, C.P. et al 2006).

abm's 3'-UTR Luciferase/GFP reporter can be used to assess the functions of the miRNA in any cell type by transducing a Luc/GFP-UTR reporter construct into your cells of interest. More often, an assay is performed by co-transfection of a 3'-UTR reporter construct and a miRNA over-expression construct (Figure 1). Any interaction between the over-expressed miRNA and its target can be evaluated by comparing reporter activities from the co-transfected samples against the samples transfected with a blank control vector (lacking the 3'-UTR insert).

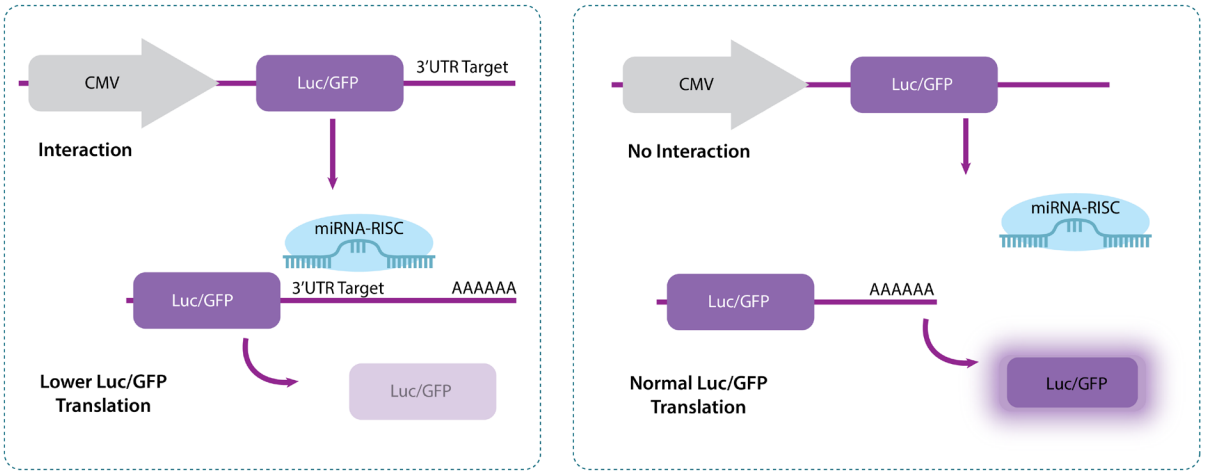

Figure 2: Methodology behind target gene 3'-UTR reporters in validating specific miRNA interactions. miRNAs that specifically bind to the 3'-UTR region of a target gene mRNA transcript will inhibit the downstream translation of the Luc/GFP reporter mRNA into protein.

ii. Promoter Reporter Vectors

Our Promoter Reporter Vectors offer a huge variety of human and mouse promoters driving either GFP or Luciferase. We include the range of approximately -1500 bp upstream to +100 bp downstream of the transcription start site.

One common way to use promoter reporter constructs is to subject cells carrying the construct to a stimulus of interest and observe how this affects reporter expression. Or, one can perform an expression assay with different promoter variants to observe how changes in the promoter sequence affect gene expression.

We recommend always performing your experiments with the proper controls. To this end, we offer a blank promoter reporter vector (negative control), and CMV promoter reporter vector (positive control).

iii. 5′ UTR Reporter Vectors

Every gene has a 5′ UTR and 3′ UTR (Untranslated Region) upstream and downstream, respectively, of the coding region. While 3′ UTRs are the major focus of regulatory research, it's been found that 5′ UTRs also mediate translational regulation via elements such as upstream start codons (uAUGs), upstream open reading frames (uORFs), secondary structures, RNA binding protein (RBP) binding motifs, and miRNA binding.

abm's 5′ UTR Reporter Vectors consist of the 5′ UTR of the gene of your choice preceding a GFP or Luciferase reporter, driven by a β-actin promoter. These vectors are ideal for studying the regulation of translation by 5′ UTR elements. The 5′ UTR reporter constructs can be combined with our Site-Directed Mutagenesis Service (Cat. No. C047) in order to define RBP or miRNA binding sites, or investigate the effects of uAUGs or uORFs on gene expression. To use alongside our custom 5′ UTR Vectors, we offer a blank 5′ UTR Vector (negative control).

C. Protocols

i. General Transfection Protocol

1. DNA seed stock should first be transformed and amplified in a DH5a bacteria strain and selected with Kanamycin. It is advisable to perform a maxi preparation to obtain enough DNA for future applications.
2. One day before transfection, seed 293 cells at a density of 70% in a 6 well plate.
3. The following day, set up the transfections using a standard transfection protocol. Refer to **abm** Cat. No. G2100.
4. The same amount and quality of DNA should be used for each transfection. Any difference in the amount of DNA used can be compensated by adding a common plasmid (such as pShuttle, A001). For reporter and microRNA construct co-transfection, adjust the ratio to 2:1 (miRNA Vector: UTR Reporter Vector).

ii. Generation of UTR Cell Lines

An added capability of **abm**'s UTR constructs is generating stable cell lines through lentiviral transduction to enable expression of a specific UTR. This is particularly useful for difficult-to-transfect primary cells. Alternatively, you can purchase one of our pre-made 3'UTR Reporter Cell Lines. With a stable UTR GFP/Luc expressing cell line, a library of miRNAs can easily be screened by transfection or lentivirus infection methods.

1. Subculture the target cells in a 6-well plate to a 70% density. Determine an optimal killing curve for the cells by adding Puromycin to the growth medium a range of concentrations between 0.1 and 1 µg/ml.
2. Generate a high titre lentivirus using the chosen construct (refer to Lentivirus Packaging Protocol, https://www.abmgood.com/misc/pdfs/Lenti-Packaging.pdf) and transduce the target cells (refer to Lentivirus Infection Protocol, https://www.abmgood.com/misc/pdfs/Lenti-Infection.pdf).
3. Subculture cells from a 6 well plate to two or three 10 cm dishes at different cell densities for stable clone selection by adding the optimal concentration of puromycin as determined by the killing curve assay from step 1.
4. It is critical to have a control condition carried out at the same density of cells without lentivirus transduction, but with puromycin selection. Complete cell death in the control dish compared with cell survival in transduced conditions indicates successful selection.
5. Once selection is completed, optimal stable clones can be screened by luciferase assay or fluorescent microscopy.

iii. Assay for Endogenous miRNA Expression With Transient Transefction of 3' or 5' UTR Reporter

1. Prepare the DNA and set up the transfection as indicated in Section C.i. - *General Transfection Protocol*.
2. The following day, set up the transfection using a standard transfection protocol (refer to **abm** Cat. No. G2100 for protocol). The experimental design in the table below can be used as a general guide:

Transfection	Control 1	Control 2	Experiment
Blank UTR Control Vector	+	-	-
5' UTR or 3' UTR Reporter Vector	-	+	+
pLenti-miRNA Inhibitor*	-	+	-

*Available from our miRNA library, or custom-made.

3. 48-72 hours after transfection, assay reporter expression using fluorescent microscopy, Western Blot, or a luciferase assay kit (**abm** Cat. No. G287).

When co- transfected with an miRNA inhibitor, an attenuated or abolished interaction between the miRNA and the UTR of the Reporter Vector should be observed.

4. The interactions between an miRNA and UTR can be further validated by mutating regions within the UTR sequence. For this purpose, the below set up can be used.

Transfection	Control	Exp. 1	Exp. 2	Exp. 3	Exp. 4
pLenti-Luc/GFP Control	+	-	-	-	-
pLenti-Luc/GFP-UTR	-	+	-	+	-
pLenti-Luc/GFP-UTR Mutant**	-	-	+	-	+
pLenti-miRNA Inhibitor*	-	-	-	+	+

**Available using our custom Site-Directed Mutagenesis Service (C047)

5. Prepare the DNA and set up the transfection as indicated in Section C.
6. 48-72 hours after transfection, assay reporter expression using fluorescent microscopy, Western Blot, or a luciferase assay kit (**abm** Cat. No. G287).

Wildtype UTR reporters should show less reporter activity than mutant UTR reporters if the miRNA would normally bind to the mutated region.

iv. Assay for Exogenously Expressed miRNA and Target 3' or 5' UTR Interactions With Transient Transfection

To assay for interactions between an exogenously expressed miRNA and a 3'-UTR Luc/GFP Reporter construct, we suggest setting up a screening experiment using **abm**'s pLenti-miRNA vectors and pLenti-Luc/GFP-UTR vectors. The same amount and quality of DNA should be used for each transfection. An RFP control plasmid can be used for normalizing the variation between transfections.

1. Prepare the DNA and set up the transfection as indicated in Section C.i. - *General Transfection Protocol*.
2. The experimental design shown in the table below can be used a general guide:

Transfection	Control	Experiment
pLenti-miRNA Blank Control	+	-
5' UTR or 3' UTR Reporter Vector	+	+
pLenti-miRNA*	-	+

*Available from our miRNA library, or custom-made.

An interaction between the miRNA and UTR of the Reporter Vector will result in lower reporter activity than the no-miRNA control.

3. When positive interactions are identified, further validation should be carried out in the following configurations.

Transfection	Control	Exp. 1	Exp. 2
pLenti-miRNA Blank Control	+	-	-
5' UTR or 3' UTR Reporter Vector	+	+	-
5' UTR or 3' UTR Reporter Mutant**	-	-	+
pLenti-miRNA	-	+	+

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4. 48-72 hours later, assay reporter expression using fluorescent microscopy, Western Blot, or a luciferase assay kit (**abm** cat. No. G287).

Wildtype UTR reporters should show less reporter activity than mutant UTR reporters if the miRNA would normally bind to the mutated region.

v. Assay for Promoter Variant Assessment with Transient Transfection

1. Prepare the DNA and set up the transfection as indicated in Section A.
2. The experimental design shown in the table below can be used a general guide:

Transfection	Control	Exp. 1	Exp. 2	Exp. 3
Blank Promoter Reporter Lentiviral Vector	+	-	-	-
Promoter Reporter Vector	-	+	-	-
Promoter Reporter Variant 1*	-	-	+	-
Promoter Reporter Variant 2*	-	-	-	+

*Available using our custom Site-Directed Mutagenesis Service (C047)

3. 48-72 hours later, assay reporter expression using fluorescent microscopy, Western Blot, or a luciferase assay kit (**abm** cat. No. G287).

The effectiveness of the promoter variant will determine the amount of reporter activity.

vi. Assay for Promoter Reporter Response to Stimuli with Transient Transfection

1. Prepare the DNA and set up the transfection as indicated in Section A.
2. The experimental design shown in the table below can be used a general guide:

Transfection	Control	Experiment
Promoter Reporter Vector Blank	+	-
Promoter Reporter Vector	-	+

3. In parallel, perform the above experimental design with different conditions to investigate stimuli of interest (ex. low oxygen, high/low concentration of a compound, differing cell lines, etc.).

D. Frequently Asked Questions

Why should I use abm 3'-UTR reporter vectors?
abm Inc. has the largest collection of human and moe 3'-UTR vectors in a ready-to-use lentiviral format, at the most competitive pricing available. These constructs can be used either for transient transfection or stable transduction (when packaged into lentiviral particles). With lentiviral particles, difficult-to-transfect cells like primary cells can be efficiently transduced.

What are appropriate controls in a 3'-UTR reporter assay?
Based on available literature and in house testing, we believe that miRNA sponges (or 5x tandem repeats of the mature miRNA reverse complementary sequence, downstream of the luciferase or GFP stop codon in pLenti-Luc or GFP) or inhibitors can be used as excellent positive controls to validate your results.

Do I have to mutate the 3'UTR sequence to show the specificity of miRNA and its target interaction?
Yes, mutant sequences are the best way to validate that an interaction between a pLenti-miRNA and pLenti-Luc/GFP-UTR is specific. **abm** Inc. offers a custom Site Directed Mutatagenesis Service at the material cost (Cat. No. C047).

Do you offer custom lentivirus packaging service?
Yes, **abm** Inc. is a market leader in the production of recombinant lentiviral vectors and lentiviral vector packaging services. These are offered at the most competitive prices with fast turnaround times. For complete information about our services, please visit: http://www.abmgood.com/ Custom-Lentivirus-Services.html.

What is your UTR vector Guarantee?
abm Inc. warrants that each vector contains the exact sequence based on gene bank information referenced. At **abm**'s discretion, free replacement of any non-conforming product can be arranged if notified within 30 days of the product's receipt. If you experience any difficulty with an **abm** product, please contact our Technical Support Staff at 1-866-757-2414 (toll free) or at 604-247-2416 for local customers. Alternatively, email us at technical@abmGood.com for an immediate written response.

E. References

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